

AMENDMENTS TO THE SPECIFICATION

Please make the following amendments to the specification.

Replace paragraph 0028 with the following paragraph:

Reference is made to the ~~appendix submitted on six compact discs~~sequence listing submitted herewith. The ~~compact discs contain~~a sequence listing comprising 2254510 genomic sequences on~~the~~consists of a file named Seq.txt "SequenceListing.txt" (342,240342,255 KB, ~~September 28, 2005~~July 15, 2009), and is hereby incorporated by reference herein.

Replace paragraph 0290 with the following paragraph:

Fig. 23B is a schematic representation of secondary folding of hairpins of the operon-like cluster of Fig. 23A. The hairpins are associated with the following SEQ ID NOs: N2 (SEQ ID NO: 2254563); N3 (SEQ ID NO: 2254564); MIR23 (SEQ ID NO: 2254565); GAM22 (SEQ ID NO: 2254566); GAM116 (SEQ ID NOs: 2254567 and 2254568); N4 (SEQ ID NO: 2254569); N0 (SEQ ID NO: 2254570); N6 (SEQ ID NO: 2254571); MIR24 (SEQ ID NO: 2254572); N7 (SEQ ID NO: 2254573);

Replace paragraph 0292 with the following paragraph:

Fig. 24A is an annotated sequence of EST72223 (SEQ ID NO: 225462) comprising known human miRNA oligonucleotide MIR98 and novel human oligonucleotide CAM25 PRECURSOR detected by the oligonucleotide detection system of the present invention. The sequence of EST72223 includes the four marked sequences: the sequence of the miRNA-98 hairpin in bold (SEQ ID NO: 2254589), the sequence of the mature miRNA-98 in bold and underlined (SEQ ID NO: 2254590), the sequence of the GAM25 hairpin in bold (SEQ ID NO: 2254591) and the sequence of the mature miRNA of GAM25 in bold and underlined (SEQ ID NO: 2254592); and

Replace paragraphs 0458-0461 with the following paragraphs:

[0458] Fractionation was done by loading up to 500g per YM100 Amicon Microcon column (Millipore) followed by a 500g centrifugation for 40 minutes at 4C. Flow through "YM100"RNA consisting of about of the total RNA was used for library preparation or fractionated further by loading onto a YM30 Amicon Microcon column (Millipore) followed by a 13,500g centrifugation for 25

minutes at 4C. Flowthrough "YM30" was used for library preparation as is and consists of less than 0.5% of total RNA. For the both the "ligation" and the "One-tailed" libraries, RNA was dephosphorilated and ligated to an RNA (lowercase)-DNA (UPPERCASE) hybrid 5-phosphorilated, 3 idT blocked 3-adapter (5-P-uuuAACCGCATCCTTCTC-idT-3 Dharmacon # P-002045-01-05) (SEQ ID NO: 2254574) (as elaborated in Elbashir et al., Genes Dev. 15:188-200 (2001)) resulting in ligation only of RNase III type cleavage products. 3-Ligated RNA was excised and purified from a half 6%, half 13% polyacrylamide gel to remove excess adapter with a Nanosep 0.2M centrifugal device (Pall) according to instructions, and precipitated with glycogen and 3 volumes of Ethanol. Pellet was resuspended in a minimal volume of water.

[0459] For the "ligation" library a DNA (UPPERCASE)-RNA (lowercase) hybrid 5-adapter (5-TACTAATACGACTCACTaaa-3 Dharmacon # P-002046-01-05) (SEQ ID NO: 2254575) was ligated to the 3-adapted RNA, reverse transcribed with "EcoRI-RT": (5-GACTAGCTGGAATTCAAGGATGCGGTAAA-3) (SEQ ID NO: 2254576), PCR amplified with two external primers essentially as in Elbashir et al 2001 except that primers were "EcoRI-RT" and "PstI Fwd"(5-CAGCCAACGCTGCAGATACGACTCACTAAA-3) (SEQ ID NO: 2254577). This PCR product was used as a template for a second round of PCR with one hemispecific and one external primer or with two hemispecific primers.

[0460] For the "One tailed" library the 3-Adapted RNA was annealed to 20pmol primer "EcoRI RT" by heating to 70C and cooling 0.1C/sec to 30C and then reverse transcribed with Superscript II RT (According to instructions, Invitrogen) in a 20l volume for 10 alternating 5 minute cycles of 37C and 45C. Subsequently, RNA was digested with 1l 2M NaOH, 2mM EDTA at 65C for 10 minutes. cDNA was loaded on a polyacrylamide gel, excised and gelpurified from excess primer as above (invisible, judged by primer run alongside) and resuspended in 13l of water. Purified cDNA was then oligo-dC tailed with 400U of recombinant terminal transferase (Roche molecular biochemicals), 1l 100M dCTP, 1l 15mM CoCl₂, and 4l reaction buffer, to a final volume of 20l for 15 minutes at 37C. Reaction was stopped with 2l 0.2M EDTA and 15l 3M NaOAc pH 5.2. Volume was adjusted to 150l with water, Phenol: Bromochloropropane 10:1 extracted and subsequently precipitated with glycogen and 3 volumes of Ethanol. C-tailed cDNA was used as a template for PCR with the external primers "T3-PstBsg(G/I)18"(5-AATTAACCCCTCACTAAAGGCTGCAGGTGC AGGIGGGIIGGGIIGGGIIGN- 3 (SEQ ID NO: 2254578) where I stands for Inosine and N for any of the 4 possible deoxynucleotides), and with "EcoRI Nested"(5-GGAATTCAAGGATGCGGTTA-3) (SEQ ID NO:2254579). This PCR product was used as a template for a second round of PCR with

one hemispecific and one external primer or with two hemispecific primers.

[0461] Hemispecific primers were constructed for each predicted GAM RNA oligonucleotide by an in-house program designed to choose about half of the 5' or 3' sequence of the GAM RNA corresponding to a TM of about 30-34°C constrained by an optimized 3' clamp, appended to the cloning adapter sequence (for "One-tailed" libraries 5'-GGNNGGGNNG (SEQ ID NO: 2254580) on the 5' end of the GAM RNA, or TTTAACC GCATC-3' (SEQ ID NO: 2254581) on the 3' end of the GAM RNA. For "Ligation" libraries the same 3' adapter and 5'-CGACTCACTAAA (SEQ ID NO: 2254582) on the 5' end). Consequently, a fully complementary primer of a TM higher than 60°C was created covering only one half of the GAM RNA sequence permitting the unbiased elucidation by sequencing of the other half.

Replace paragraph 0482 with the following paragraph:

[0482] Transcript products were 705nt (EST72223), 102nt (MIR98 precursor), 125nt (GAM25 precursor) long. EST72223 was PCR amplified with T7-EST 72223 forward primer: 5'-TAATACGACTCACTATAGGCCCTTATTAGAGGATTCTGCT-3' (SEQ ID NO: 2254583) and T3-EST72223 reverse primer: 5'-AATTAACCCTCACTAAAGGTTTTTTTTTCCTGAGACAGAGT-3' (SEQ ID NO: 2254584). MIR98 was PCR amplified using EST72223 as a template with T7MIR98 forward primer: 5'-TAATACGACTCACTATAGGGTGAGGTAGTAAGTTGTATTGTT-3' (SEQ ID NO: 2254585) and T3MIR98 reverse primer: 5'-AATTAACCCTCACTAAAGGGAAAGTAGTAAGTTGTATAGTT-3' (SEQ ID NO: 2254586). GAM25 was PCR amplified using EST72223 as a template with GAM25 forward primer: 5'-GAGGCAGGAGAATTGCTTGA-3' (SEQ ID NO: 2254587) and T3-EST72223 reverse primer: 5'-AATTAACCCTCACTAAAGGCCTGAGACAGAGTCTTGCTC-3' (SEQ ID NO: 2254588).